

CLAIMS

Sub D1

1. A method for stably transferring DNA into multi-potential hematopoietic stem cells in the G0 phase of the cell cycle, which comprises transducing said multi-potential hematopoietic stem cells with an adeno-associated virus vector that contains said DNA.

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2. A method according to claim 1, wherein the transduced multi-potential hematopoietic stem cells are maintained under conditions such that the cells in the G0 phase do not differentiate or undergo mitosis substantially during the transduction process.

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3. A method according to claim 2, wherein the conditions under which the transduced multi-potential hematopoietic stem cells are maintained include a transduction time of about 2 hours to about 48 hours.

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4. A method according to claim 2, wherein the conditions under which the transduced multi-potential hematopoietic stem cells are maintained include a transduction time of about 2 hours to about 24 hours.

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5. A method according to claim 2, wherein the conditions under which the transduced multi-potential hematopoietic stem cells are maintained include a transduction time of about 18 hours to about 24 hours.

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6. A method according to claim 2, wherein the conditions under which the multi-potential hematopoietic stem cells are maintained include low cytokine levels.

Sub D2

7. A method according to claim 6, wherein the conditions under which the multi-potential hematopoietic stem cells are maintained include cytokine levels no greater than about 15 ng/ml IL-3, 15

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5 ng/ml IL-6 and 1.5 ng/ml granulocyte-macrophage colony stimulating factor.

8. A method according to claim ~~7~~, wherein the conditions under which the multi-potential hematopoietic stem cells are maintained include cytokine levels of about 1 ng/ml IL-3, 1 ng/ml IL-6 and 0.1 ng/ml granulocyte-macrophage colony stimulating factor to about 15 ng/ml IL-3, 15 ng/ml IL-6 and 1.5 ng/ml granulocyte-macrophage colony stimulating factor.

9. A method according to claim ~~8~~, wherein the conditions under which the multi-potential hematopoietic stem cells are maintained include cytokine levels of about 5 ng/ml IL-3, 5 ng/ml IL-6 and 0.5 ng/ml granulocyte-macrophage colony stimulating factor to about 10 ng/ml IL-3, 10 ng/ml IL-6 and 1 ng/ml granulocyte-macrophage colony stimulating factor.

10. A method according to claim ~~7~~ or 9, wherein the conditions under which the multi-potential hematopoietic stem cells are maintained include cytokine levels of about 10 ng/ml IL-3, 10 ng/ml IL-6 and 1 ng/ml granulocyte-macrophage colony stimulating factor.

11. A method according to claim ~~1~~, wherein the transduction results in stable integration of the transferred DNA into the genome of the multi-potential hematopoietic stem cells.

12. A method according to claim ~~11~~, wherein the transferred DNA is capable of remaining integrated into the genome of the multi-potential hematopoietic stem cells for at least 4 weeks.

13. A method according to claim ~~11~~, wherein the transferred gene is capable of remaining integrated into the genome of the multi-potential hematopoietic stem cells for at least 8 weeks.

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14. A method according to claim 1, wherein the multi-potential hematopoietic stem cells are CD34⁺CD38⁻ cells.

15. A method according to claim 1, wherein the adeno-associated virus vector contains said DNA within the adeno-associated virus inverted terminal repeats, and wherein the adeno-associated virus vector is encapsidated.

16. A method according to claim 1 or 14, wherein the adeno-associated virus vector is derived from the base vector ~~QWRSV~~.

17. A method according to claim 15, wherein the adeno-associated virus vector has a wild-type polyadenylation region.

18. A method according to claim 15, wherein the adeno-associated virus vector has a heterologous polyadenylation region.

Sub D4 19. A method according to claim 16, wherein the adeno-associated virus vector is vCWRHIVAPAP.

20. A method according to claim 16, wherein the adeno-associated virus vector is vCWRHIVASVN.

21. A method according to claim 16, wherein the adeno-associated virus vector is vCWRAP.

22. A method according to claim 1, wherein the DNA is selected from a gene, a gene fragment, an antisense DNA, a marker gene, a reporter gene and a recombinant DNA.

Sub B2 23. A method for stably transferring DNA into multi-potential hematopoietic stem cells in the G0 phase of the cell cycle, which comprises transducing said multi-potential hematopoietic stem cells, which comprises transducing said multi-potential hematopoietic stem cells with an adeno-associated virus vector that contains said DNA, wherein said multi-

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canv potential hematopoietic stem cells are CD34⁺CD38⁻ cells in the G0 phase of the cell cycle.

24. An adeno-associated virus vector which stably transfers DNA into multi-potential hematopoietic stem cells residing in the G0 phase of the cell cycle.

25. An adeno-associated virus vector according to claim 24, wherein the adeno-associated virus vector contains said DNA within the adeno-associated virus inverted terminal repeats, and wherein the adeno-associated virus vector is encapsidated.

26. Stably transduced multi-potential hematopoietic stem cells residing in the G0 phase of the cell cycle.

27. Multi-potential hematopoietic stem cells according to claim 26, wherein said cells substantially remain in the G0 phase of the cell cycle for at least about 2 days.

28. Multi-potential hematopoietic stem cells according to claim 27, wherein said cells substantially remain in the G0 phase of the cell cycle for at least about 7 days.

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29. Multi-potential hematopoietic stem cells according to claim 26, wherein greater than 80% of said cells remain in G0 after culture for 7 days.

30. Multi-potential hematopoietic stem cells according to claim 26, wherein said cells have been transduced with an adeno-associated virus vector.

31. Multi-potential hematopoietic stem cells according to claim 26, wherein said cells do not differentiate at an appreciable rate.

32. Multi-potential hematopoietic stem cells according to claim 31, wherein about 92% to about 99% of said cells are non-dividing after 2 days.

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33. Multi-potential hematopoietic stem cells according to claim 31, wherein about 65% to about 83% of said cells are non-dividing after 7 days.

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